# THE JUVENILE HORMONE. V. THE SENSITIVITY OF THE BUG, PYRRHOCORIS APTERUS, TO A HORMONALLY ACTIVE FACTOR IN AMERICAN PAPER-PULP [1]

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When reared in the Biological Laboratories at Harvard University, the European bug, *Pyrrhocoris apterus* L., failed to undergo normal metamorphosis. In the initial cultures, 1,215 individuals showed normal growth and moulting until the fifth larval instar. Then, instead of metamorphosing into adults, all except one individual underwent a supernumerary larval moult to form "perfect" sixth instar larvae or adultoid sixth instar larvae (Table I). All the perfect sixth instar larvae underwent yet another supernumerary moult to form even larger seventh instar larvae or seventh instar adultoid larvae. Of the entire group of 1,215 larvae, only one individual completed metamorphosis and attained sexual maturity.

Among tens of thousands of *Pyrrhocoris* which one of us (K. S.) had cultured in Prague, the spontaneous formation of a sixth instar had never been encountered. Metamorphosis had always taken place at the end of the fifth larval stage—a result directly attributable to the inactivation of the corpora allata and the cessation of juvenile hormone secretion at the outset of the fifth instar (Sláma, 1962). The formation of sixth stage larvae had been provoked only in experiments in which juvenile hormone was supplied by the implantation of active corpora allata (Novák and Sláma, 1961; Sláma, 1964a). For these several reasons, it seemed certain that the Harvard cultures of *Pyrrhocoris* had access to some unknown source of juvenile hormone.

One possibility was that some feature of the Harvard environment served to prevent the normal inactivation of the animal's own corpora allata and that, in this indirect manner, metamorphosis was blocked by the prolonged and abnormal secretion of juvenile hormone. An alternative possibility was that the Harvard cultures were under the influence of an environmental factor which, in itself, possessed juvenile hormone activity.

The present study initiates a detailed investigation of the phenomenon.

## Materials and Methods

### 1. *Experimental animals*

*Pyrrhocoris apterus* (Heteroptera; Pyrrhocoridae) is a typical hemipteran found throughout Europe feeding by its sucking mouth-parts on the seeds of lime

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(linden) trees (*Tilia* sp.). The insect is not known to occur in the United States or Canada.

A subculture of *Pyrrhocoris apterus* was obtained from the Prague Laboratory of Insect Physiology and brought to Harvard by permission of the United States Department of Agriculture. The experimental cultures were reared in 10-cm Petri dishes in a 25° C incubator programmed for a daily illumination of 17 hours. Each Petri dish contained a supply of linden seed (*Tilia cordata*), along with a cotton-plugged vial of tap water. To increase the surface available to the bugs, each dish also contained a folded strip of paper towelling (Scott Paper Co., Brand 150).

Certain experiments were performed on two other species of Heteroptera—*Rhodnius prolixus* and *Oncopeltus fasciatus*. *Rhodnius* was obtained from Dr. Andrew Spielman, of the Harvard Medical School, and Mr. Paul Toselli, of the Yale Medical School; *Oncopeltus* was supplied by Prof. A. Glenn Richards, of the University of Minnesota.

TABLE I

*Supernumerary larval instars in the Harvard cultures of Pyrrhocoris apterus*

| 5th instar larvae moulted into: | Number of animals |
|---|---|
| Perfect or almost perfect 6th instar larvae* | 500 |
| Adultoid 6th instar larvae | 714 |
| Normal adults | 1 |

* Many individuals underwent a further moult to form 7th instar larvae or adultoids.

Certain other experiments were performed on five species of silkworm pupae, namely, *Hyalophora cecropia*, *H. gloveri*, *Antheraea polyphemus*, *A. mylitta*, and *Samia cynthia*. The first three species were stored at 5° C; the last two at 8° C. In order to induce adult development, the previously chilled pupae were placed at 25° C.

### 2. *Allatectomy*

Corpora allata were removed from *Pyrrhocoris* by the technique described by Sláma (1964b).

## RESULTS

### 1. *Anatomical and behavioral studies*

Of the large number of abnormal *Pyrrhocoris* reared at Harvard, a total of 435 were subjected to detailed examination and dissection. The results were as follows:

Those sixth instar larvae which showed no trace of adult characters underwent a second supernumerary moult after five further days. The seventh instar larvae were often unable to escape from the exuviae and, in that event, they soon died. The females, on dissection, showed juvenile ovaries without any ripened eggs; by contrast, the seminal vesicles of the males were full of mature spermatozoa.

Those sixth instar larvae which showed only a few patches of adult cuticle

also tried to moult again. However, the old cuticle remained attached to the epidermis at the sites of the patches of adult cuticle. Consequently, all these individuals died without completing the ecdysis.

The adultoid sixth instar larvae behaved in one of two ways. In the process of moulting to the sixth instar, the majority soon died without being able to detach the exuviae from the appendages or other parts of the body. A lesser number completed the ecdysis and then survived for one to three months without any further moulting. On dissection, the thoracic glands were found partly or wholly degenerate. Here, again, the males showed mature spermatozoa, while, surprisingly enough, the females contained ripe, chorionated eggs which, in the absence of functional genitalia, could not be oviposited. Since feeding is impossible when the abdomen is distended with ripe eggs (Sláma, 1964c), all these females died within a month or so.

The adultoid sixth instar larvae showed bizarre behavior. Despite the absence of external genitalia, the adultoid females were extremely attractive to the adultoid males and the latter made repeated efforts to copulate. So also, the adultoid males pursued and tried to mate with normal females, while normal males tried to mate with adultoid females. Needless to say, all these efforts were unsuccessful in the absence of genitalia. These findings point to the secretion of sex pheromone by the adultoid females and the maturation of adult sexual behavior by the adultoid males.

### 2. *The environmental factor*

A detailed comparison of the culture conditions at Harvard *versus* Prague suggested 15 differences. Attention first centered on the fact that the Harvard cultures were stored in incubators which also contained silkworm pupae at various stages of adult development. We were mindful of Loher's (1960) finding that the activity of the corpora allata of the desert locust can be sustained by a volatile emanation from other locusts. It seemed altogether likely that we had encountered an analogous interaction between *Pyrrhocoris* and the developing silkworms. Appropriate experiments were performed to test this hypothesis. All were negative.

We also examined the possibility that the Petri dishes, or the seed, or the water-vials, or the water itself had somehow become contaminated with one or more of the potent preparations of juvenile hormone under simultaneous study in the Harvard laboratory. No evidence could be found to support any of these propositions.

By a process of elimination, attention finally focused on the fragment of paper towelling which had been placed in each Petri dish. In Prague, filter paper had routinely been used in the cultures. We were astonished to find that when the towelling was replaced by a corresponding fragment of Whatman's filter paper, the entire phenomenon vanished and all individuals developed normally.

### 3a. *Scott, Brand 150*

The just-mentioned finding seemed incomprehensible. In correspondence with the Philadelphia laboratory of the Scott Paper Company, we sought to inform

ourselves as to the chemicals added to Brand 150. All we learned was that the towelling was made from paper-pulp.

3b. *Tests of other paper products*

Twenty other brands of towelling, napkins, and bathroom tissues were assembled and tested by allowing freshly moulted, fifth instar *Pyrrhocoris* larvae to walk upon them. The following brands showed high juvenile hormone activity as signaled by the moulting of all individuals into sixth instar larvae or adultoid forms: *Scottowels, Scott Tissue Service Roll, Scott Cut-Rite Wax Paper, Scotties, Scotkins Paper Napkins, Kleenex, Kleenex Two-Ply Tissues, Kleenex Bathroom Tissues, Kleenex Dinner Napkins, Hudson Table Napkins, Kimwipes, Doeskin Facial Tissues.*

These extraordinary findings suggested that the active factor was widespread in paper products. Therefore, additional tests were performed on newspapers and journals. In each case, a fragment of the material was placed in the culture dishes in contact with freshly moulted, fifth instar *Pyrrhocoris* larvae.

The *New York Times, Wall Street Journal, Boston Globe, Science,* and *Scientific American* proved to be even more active than the above-mentioned towelling. By contrast, the *London Times* and *Nature* were completely inert, and so were a number of other paper materials of European or Japanese manufacture. Paradoxically, Japanese newspapers were as active as American ones. However, we later learned that Japanese newsprint is imported from Canada.

The conclusion was self-evident: American paper products contain an active material which blocks the metamorphosis of *Pyrrhocoris.* European or Japanese paper products are apparently deficient in this factor.

4. *The active principle*

Paper towels were cut up with scissors, placed in beakers, and soaked in water, methanol, acetone, and diethyl ether, respectively. After an hour the solvents were decanted and filtered. Discs of Whatman's filter paper were dipped into each extract and then dried. The impregnated papers were placed in Petri dishes along with seven freshly moulted, fifth instar *Pyrrhocoris* larvae.

Five days later, all the larvae exposed to the methanol, acetone, or ether extracts moulted into sixth instar larvae. By contrast, the larvae exposed to the aqueous extract transformed into normal adults after a total of seven days.

The solvent was evaporated from the acetone extract to obtain a crude preparation of the material. The latter was heated in a boiling water bath for 10 minutes, redissolved in acetone, and impregnated onto discs of filter paper for testing. Full activity was retained, thereby demonstrating that the active material is heat-stable.

Another preparation of active extract was dissolved in 2 ml of methanol, mixed with 1 ml 50% KOH, and saponified for 15 minutes at 50° C. After the extraction of the non-saponifiable fraction with ether, the mixture was acidified and the saponifiable fraction extracted with ether. Both fractions were inactive when impregnated onto filter paper and assayed in the usual manner. This shows that the active factor is destroyed by treatment with alkali.

5. *Partial purification of the crude extract*

A hundred towels (*Scott, Brand 150*) were extracted with a total of 4 liters of absolute methanol. The extract was decanted, filtered, and the solvent evaporated on a rotary evaporator; wt 2.32 g.

The crude material was extracted with four 25-ml changes (hereafter, 4 × 25-ml) of petroleum ether (bp 30–60° C). The solvent was decanted and evaporated on a warm plate to yield a golden oil along with a greenish precipitate; wt 1.42 g. The oil solidified on cooling.

The material insoluble in petroleum ether was extracted with 4 × 25 ml benzene to obtain a green immobile film; wt 0.14 g. Further extraction with 4 × 25 ml diethyl ether gave an unweighable white film. Final extraction with 4 × 25 ml absolute methanol provided a pale yellow, amorphous material; wt 0.65 g.

Each of the four fractions was dissolved in acetone, impregnated onto filter paper, and assayed on *Pyrrhocoris*. All the activity was recovered in the initial petroleum ether fraction.

6. *Silicic acid chromatography*

The petroleum ether fraction was dissolved in 100 ml petroleum ether (bp 30–60° C) and filtered to get rid of the insoluble, green sediment. About 60 ml of this solution was percolated onto a 2-cm (ID) column composed of a slurry of 30 g silicic acid ("Unisil") prepared in petroleum ether. The column was eluted successively with 125 ml of petroleum ether; petroleum ether-benzene 1:1; pure benzene; benzene-diethyl ether 1:1; pure ether; and absolute methanol. Assays of the several fractions showed that nearly all the activity was recovered in the fraction eluted in equal parts of benzene and diethyl ether; wt 0.28 g.

Solvent extraction and chromatography therefore achieved an approximately four-fold purification of the original, highly active methanolic extract.

7. *Botanical sources of the active material*

The presence of the active substance in so many American paper products suggested that the factor was derived from one or more species of American pulp trees.

Through the kindness of Professor Irving W. Bailey, authentic samples of seven species of gymnosperms were obtained from the wood collection of the Harvard Herbarium (where the specimens had been stored for 25 or more years). Each specimen was first rinsed in acetone and then cut up on a drill press with an acetone-washed drill. The pulverata were shaken with acetone, filtered, and the solvent evaporated to obtain the acetone-soluble materials. The latter were dissolved in small volumes of methanol, impregnated onto filter paper, and assayed on *Pyrrhocoris*.

High activity was recorded for extracts of balsam fir (*Abies balsamea*), hemlock (*Tsuga canadensis*), yew (*Taxus brevifolia*), and American larch (*Larix laricina*). Extracts of the southern pine (*Pinus echinata*) and European larch (*Larix decidua*) showed only traces of activity, while extracts of red spruce (*Picea rubra*) appeared to be inactive.

These findings point to botanical origins of the active material. Evidently, the substantial activity in American paper products is mainly derived from balsam fir (*Abies balsamea*)—a principal pulp tree indigenous to America. The negative tests recorded for paper samples of European and Japanese manufacture suggest that the pulp trees of Europe and Japan do not contain the active principle.

### 8. *The entry of the active material into the insect*

Freshly moulted, fifth instar larvae, together with food and water, were placed in a glass jar and the latter capped with copper netting. This jar was placed in a sealed one-gallon jar containing a number of paper towels.

In the absence of any contact with the towelling, all individuals transformed into normal adults. This showed that the active factor is not volatile at room temperature and that actual contact with the factor is necessary.

We supposed that the point-of-entry might be the thin, elastic cuticle of the pulvillus at the tip of each leg. However, when all of the pulvilli were amputated, the bugs were no less sensitive to the towelling.

On close inspection, we found that all individuals touched the paper with their antennae from time to time. Therefore, it seemed possible that the antennae were the points-of-entry. To test this possibility, a piece of copper netting was placed on the towelling and a second netting supported 1 mm above the first. Freshly moulted fifth instar larvae were reared on the second netting so that they could touch the paper with the antennae but not with the legs. All these individuals showed a positive response by moulting into adultoid forms.

In this case the site of entry was, unquestionably, the antennae. However, in other experiments we found that when the antennae were amputated, the larvae remained fully sensitive to direct contact with the towelling.

All these observations suggest that the active material can gain entry through any part of the integument. This hypothesis was examined as soon as the active material was available as an extract.

By means of a micro-syringe, a droplet (*ca.* 0.1 $\mu$l) of an acetone solution of partially purified extract was applied to specific body regions in each of a large series of freshly moulted fifth instar larvae. The extract proved to be fully effective when placed on any part of the larva—even at the tip of a leg or antenna. This demonstrated that the active material readily penetrates the intact cuticle and is then translocated throughout the body.

The extract was also fully effective when injected. This was true even when precautions were taken to avoid any contact between the extract and the outside of the cuticle or epidermis.

When diluted with acetone, the extract remained fully effective when topically applied. However, when dissolved in peanut oil, the effects were more or less localized to the site of application.

### 9. *Experiments on allatectomized larvae*

The juvenile hormone activity of the extract was documented in a direct manner in experiments performed on 17 larvae from which the corpora allata had been removed at the outset of the fifth instar. Each individual then received a

TABLE II

*Effects of contact with partially purified extract impregnated on filter paper**

| Purified extract on filter paper (mg) | Number of individuals | 5th instar larvae moulted into: |
|---|---|---|
| 1.0 | 7 | Perfect 6th instar larvae |
| 0.5 | 7 | Perfect 6th instar larvae |
| 0.25 | 7 | Nearly perfect 6th instar larvae |
| 0.125 | 6 | Adultoid 6th instar larvae |
| 0.062 | 7 | Adultoid 6th instar larvae |

* Each disc of filter paper was 9 cm in diameter.

topical application of purified extract. Five days later, all individuals moulted into sixth instar larvae or adultoid forms. In each of the 17 individuals, the absence of corpora allata was confirmed by dissection. These findings demonstrated that the active principle is, in fact, a potent analogue of the juvenile hormone of *Pyrrhocoris apterus*.

10. *Quantitative studies*

A weighed sample of the partially purified extract described in Section 6 was serially diluted in acetone and used to impregnate five 9-cm discs of Whatman's No. 1 filter paper with a known amount of extract. Each disc was placed in a Petri dish along with 6 or 7 freshly moulted, fifth instar larvae.

The results are summarized in Table II. After five days all individuals moulted into sixth instar larvae or adultoid forms. Perfect sixth instar larvae were obtained when the paper contained as little as 500 $\mu$g of extract. The lowest dose tested (62 $\mu$g) was still effective in causing the formation of adultoid forms.

In order to appraise the absolute sensitivity of the bugs, a measured microdrop of the acetone solution was applied to the dorsum of each of 36 freshly moulted, fifth instar larvae. The results, summarized in Table III, show that the lowest dose tested (0.01 $\mu$g) caused all individuals to moult into adultoid forms.

11. *Effects on other species*

We were astonished to find that our most active extracts were without any detectable effects when injected into previously chilled pupae of the *polyphemus* silkworm—even when tested in the ultra-sensitive wax-wound assay for juvenile

TABLE III

*Effects of topical application of purified extract to 5th instar Pyrrhocoris*

| Amount applied ($\mu$g) | Number of individuals | 5th instar larvae moulted into: |
|---|---|---|
| 10 | 10 | Perfect 6th instar larvae |
| 1 | 7 | Perfect 6th instar larvae |
| 0.1 | 10 | Adultoid 6th instar larvae |
| 0.01 | 9 | Adultoid 6th instar larvae |

hormone (Schneiderman and Gilbert, 1958; Williams and Law, 1965). The same negative results were obtained on all the other silkworm pupae available to us: *Hyalophora cecropia, H. gloveri, Antheraea mylitta,* and *Samia cynthia.*

Even more surprising was the finding that the extract was without any effects on two other species of Heteroptera, *Rhodnius prolixus* and *Oncopeltus fasciatus.* These species were also insensitive to prolonged contact with towelling or with filter paper impregnated with active extract.

Additional experiments have demonstrated that few species are sensitive to the towel extract. Indeed, until recently, the only sensitive species had proved to be *Pyrrhocoris apterus,* itself.

12. *Relation of the extract to juvenile hormone*

The extract, as we have seen, shows high juvenile hormone activity when tested on *Pyrrhocoris* and no activity when assayed on *Oncopeltus.* This strange finding was further explored by transplanting active, adult corpora allata into freshly moulted fifth instar larvae of the two species. The adult glands of *Oncopeltus* were fully effective in blocking the metamorphosis of *Pyrrhocoris;* so, also, the adult corpora allata of *Pyrrhocoris* blocked the metamorphosis of *Oncopeltus.*

From these experiments we learn that juvenile hormone is completely interchangeable between the two species. Despite this fact, the paper extract mimics the juvenile hormone of *Pyrrhocoris,* but not of *Oncopeltus.*

13. *Effects of cecropia juvenile hormone on Pyrrhocoris*

A partially purified preparation of *cecropia* oil was utilized which, as previously described (Williams and Law, 1965), routinely shows marked juvenile hormone activity when 5 mg are injected into pupae of *Antheraea polyphemus* (weight 5 g) or into other saturniids. Fifth instar *Pyrrhocoris* weigh 15 to 45 mg and are, therefore, only 1% to 3% the mass of the silkworm pupae. So, if *Pyrrhocoris* is fully sensitive to the juvenile hormone of *cecropia,* the effective dose should be about 0.1 mg.

Five times this dose was injected into each of a series of freshly moulted, fifth instar *Pyrrhocoris* larvae. The hormone had no detectable effects and all individuals moulted into normal adults.

In another experiment, *Pyrrhocoris* larvae were reared in contact with a disc of filter paper impregnated with 25 mg of *cecropia* extract. All individuals underwent normal metamorphosis.

These findings confirm previous observations that the juvenile hormone of the *cecropia* silkworm has little or no effects on *Pyrrhocoris* (Sláma, 1961; 1962). And yet, strange to say, the *cecropia* hormone is effective when injected (Williams, 1956) or topically applied (Wigglesworth, 1958) to *Rhodnius.*

## Discussion

1. *The relation of the paper factor and juvenile hormone*

As summarized in Table IV, *Pyrrhocoris* is sensitive, not only to the juvenile hormone of *Oncopeltus,* but also to the paper factor. *Oncopeltus* is sensitive to the juvenile hormone of *Pyrrhocoris,* but insensitive to the paper factor. So, in terms

of its inactivity for *Oncopeltus,* the paper factor fails to duplicate all the biological effects of *Pyrrhocoris* hormone. This suggests that, despite its high activity for *Pyrrhocoris,* the paper factor is a different molecule from the authentic juvenile hormone of *Pyrrhocoris apterus.*

2. *The relation of farnesol and juvenile hormone*

Table IV also illustrates the marked differences encountered when the terpenoid alcohol, farnesol, is assayed on diverse species. For certain species, such as *Tenebrio* (Schmialek, 1961), farnesol possesses high juvenile activity. But in assays on many other species—for example, *Pyrrhocoris, Oncopeltus, H. cecropia,* and *A. polyphemus*—farnesol shows little or no activity. This demonstrates that farnesol can mimic the juvenile hormone of certain species but not of others.

TABLE IV

*Sensitivity of five species to juvenile hormones and mimetic substances*

| Substance | Species of insect | | | | |
|---|---|---|---|---|---|
| | *Pyrrhocoris* | *Oncopeltus* | *Rhodnius* | *A. polyphemus* | *H. cecropia* |
| *Pyrrhocoris* hormone | +++++ | +++++ | ? | ? | ? |
| *Oncopeltus* hormone | +++++ | +++++ | ? | ? | ? |
| *Cecropia* hormone | +? | +? | +++ | +++++ | +++++ |
| Farnesol | + | + | +++ | + | + |
| Paper extract | +++++ | 0 | 0 | 0 | 0 |

3. *The relation of cecropia extract and juvenile hormone*

Attention is now directed to the juvenile hormone activity of extracts prepared from the abdomens of male *cecropia* moths. The crude extract (Williams, 1956; Gilbert and Schneiderman, 1960), as well as purified fractions (Schneiderman and Gilbert, 1964; Williams and Law, 1965), show high activity in "pupal assays" performed on *cecropia, polyphemus,* and *cynthia.*. But, as indicated in Table IV, little or no activity is evident in tests performed on *Pyrrhocoris* or *Oncopeltus.* This implies that the *cecropia* hormone is chemically different from *Pyrrhocoris* or *Oncopeltus* hormone.

4. *Chemical diversification of juvenile hormone*

All these observations argue in favor of the existence of more than one molecular species of juvenile hormone. Evidently, during the millions of years of insect evolution, the detailed chemistry of the hormone has also evolved and diversified. So, today, there appears to be a number of related molecules—presumably terpenoid in character—which serve as juvenile hormone for diverse insects.

5. *Diversification of the receptor mechanisms*

Evolutionary changes in the chemistry of juvenile hormone would, of necessity, proceed hand-in-hand with homologous changes in the receptor mechanisms in the

cells and tissues. It seems altogether likely that this biochemical "retuning" of the target organs includes steric changes in the hypothetical "receptor sites" to accommodate the altered conformation of the hormone.

The probable significance of these changes in the target organs may be illustrated in the case of insects injected with farnesol. In a highly sensitive species such as *Tenebrio,* the detailed properties of the cellular mechanism permit the type of binding prerequisite for full endocrine activity. Presumably, in the case of a less sensitive species such as *Rhodnius,* the binding is less effective. And in the case of insects insensitive to farnesol, one may conjecture that the molecule is excluded from effective binding to the hormone receptors.

### 6. *The paper factor as an analogue of Pyrrhocoris hormone*

As reported in the section on Results, two lines of evidence suggest that the paper factor acts directly on the tissues of *Pyrrhocoris.* Thus, as we have seen, the extract is fully effective when assayed on allatectomized larvae. Moreover, when the extract was dissolved in peanut oil and topically applied, its juvenilizing effects remained more or less localized.

For these several reasons, the paper factor appears to be a potent analogue of *Pyrrhocoris* hormone which acts directly on the target tissues to provoke the same reactions as the native hormone, itself.

### 7. *The insensitivity of Rhodnius and Oncopeltus to the paper factor*

We have emphasized that, up to the present time, few species have been found sensitive to the paper extract. The insensitivity of the saturniids (Table IV) is not difficult to comprehend since the separate evolutions of the Lepidoptera and Heteroptera have been going on for at least 200 million years (Prof. F. M. Carpenter, personal communication). The paradox is the insensitivity of *Rhodnius* and *Oncopeltus*—two other species of Heteroptera—to the paper factor.

One possibility is that *Rhodnius, Oncopeltus,* and most other insects possess a mechanism for metabolizing or inactivating the paper factor; for obvious reasons, this mechanism would either be absent or ineffective in the case of *Pyrrhocoris.* In a subsequent communication, we shall present evidence which strongly argues against this hypothesis.

A second possibility has already been considered in connection with the differential sensitivity to farnesol—namely, that in the vast majority of insects the conformations of the receptor sites for juvenile hormone exclude the paper factor from the type of effective, catalytic binding which it promptly achieves with the target organs of *Pyrrhocoris.* We have a high regard for this theory.

A further possibility is that the hormonal receptors are intracellular and that the paper factor is able to enter the cells of *Pyrrhocoris,* but, for some unexplained reason, is unable to enter the cells of *Oncopeltus.* We are unenthusiastic about this proposition.

A final possibility is that the paper factor is selectively bound, but is inactive in *Oncopeltus* and other insensitive species. Under this circumstance the paper factor might be a potent inhibitor of the juvenile hormone. We have been unable to demonstrate any such effects.

A decision among these several hypotheses seems so important that the entire phenomenon is being studied in further detail.

### 8. *The paper extracts as an insecticide*

The paper factor is little short of a pyrrhocoricide. As reported in the section on Results, the application of 0.01 μg of the partially purified extract is effective in blocking the normal metamorphosis or sexual maturation of fifth instar larvae. One gram of the extract would therefore be sufficient to "kill" 100 million of these bugs on contact.

Chemical studies performed in collaboration with Prof. John H. Law suggest that the partially purified extract contains only a small concentration of the active principle—certainly not in excess of a few per cent. So, the absolute sensitivity of fifth instar *Pyrrhocoris* larvae must be measured in fractions of millimicrograms.

The present investigation has therefore confirmed an earlier suggestion that juvenile hormone is potentially an effective insecticide (Williams, 1956). What was not then appreciated is the apparent diversification, not only of the detailed chemistry of the hormone, itself, but also of the receptor mechanisms at the cellular level. These prospects point the way to the use of selective agents to control selected pests.

Though *Pyrrhocoris apterus* is a benign insect, we have elsewhere (Sláma and Williams, 1965) called attention to the fact that certain other Pyrrhocoridae are major pests, especially in Asia. This we can state with assurance: any species of insect which shares the endocrine sensitivities of *Pyrrhocoris apterus* is subject to control, if not eradication, by the active principle available on an unlimited scale in American paper products.

### 9. *Insects and the gymnosperms*

All the evidence points to the synthesis of the active factor in the paper extract by the balsam fir (*Abies balsamea*) and certain other species of gymnosperms. By this particular synthesis, the balsam fir and its allies have achieved full protection against any insect which shares the endocrine sensitivities of *Pyrrhocoris apterus*.

The most intriguing possibility is that the active factor in the balsam fir is a "biochemical memento" of the juvenile hormone of a former natural enemy of these trees—a predator which, for obvious reasons, is extinct, or has shifted its diet to other plants, or has survived in areas where these trees do not occur.

There is reason to believe that the active factor in the paper extract is some sort of terpene derivative; indeed, several compounds of this general type have been found to exhibit detectable juvenile hormone activity for *Pyrrhocoris* (Sláma and Williams, unpublished observations). What may be the significance of the vast number of terpenes and terpenoids which the gymnosperms synthesize for no apparent reason? Is it possible that, in the absence of any dependency on insects for pollination, the gymnosperms have evolved an incredibly sophisticated self-defense against insect predation—a method of insect control which we are just beginning to comprehend?

## Summary

1. Materials composed of American paper-pulp contain an extractable, heat-stable lipid which exhibits extremely high juvenile hormone activity when injected or topically applied to the European bug, *Pyrrhocoris apterus*. The active principle in the paper materials is derived from certain species of pulp trees, more particularly the balsam fir, *Abies balsamea*. Larvae exposed to the active material ultimately die without completing metamorphosis or attaining sexual maturity.

2. Despite its extremely high activity for *Pyrrhocoris apterus,* the extract is without any detectable effects on silkworm pupae or most other species of laboratory insects; conversely, juvenile hormone extracts prepared from *cecropia* silkmoths show little or no activity when tested on *Pyrrhocoris*. These findings point to a diversification of the detailed chemistry of juvenile hormone and to corresponding changes in the conformation of the receptor mechanism at the cellular level.

3. The factor extracted from paper materials promises to be an effective agent for the selective control of insect pests which show the same endocrine sensitivities as *Pyrrhocoris apterus*. It is possible that hormonally active factors for other species may be present in other gymnosperms.

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